Utility of Genomics and HTS Approaches for the Assessment of Industrial Chemicals and Pesticides

Philip Sayre, Ph.D.
Risk Assessment Division
Office of Pollution Prevention and Toxics
U.S. EPA (Washington, DC)
sayre.phil@epa.gov

Overview

- **Numerous chemicals are subject to EPA oversight**
- **Current Agency Guidance on 'Omics**
- **ToxCast and Industrial Chemicals' Evaluations, including High Production Volume Chemicals**
- **ToxCast and Pesticides**
- **Current Examples of Omics Use in Individual Chemicals' Assessments**

Examples of Chemicals Under Consideration by EPA

- Industrial Chemicals (TSCA Inventory has over 83,000)
  - High Production Volume Challenge Chemicals: Data now in hand on 800 chemicals (of ~ 1,400) in HPVIS
  - New chemicals (~ 1,500 Notices/yr)
- Pesticides (5000 regulatory decisions annually)
  - Pesticide Actives
  - Pesticide Inerts
  - Antimicrobials
- Drinking Water Contaminants on the Contaminant Candidate List: ~ 42 chemicals
- Toxic Release Inventory: Almost 600 chemicals

Possible Advantages of Genomics & HTS Approaches

- Make existing animal testing more focused on risk assessment/management needs
  - Guiding work on an individual chemical
  - Prioritizing work for groups of chemicals
- Reduce / refine / replace animal usage
- Reduce cost and time for data development
- Reduce cost and time for EPA review of data

EPA Interim Genomics Policy

- **Broadly describes genomics to include DNA, mRNA, and protein analyses (www.epa.gov/osa/spc/genomics.htm)**
- **Encourages and supports genomics research for understanding the molecular basis of toxicity and developing biomarkers of exposure, effects and susceptibility**
- **Genomics data alone are currently insufficient as a basis for risk assessment and management decisions**
- **May be used in a weight-of-evidence approach for human health and ecological risk assessments**

Selected Genomics Task Force White Paper views on Regulatory & Risk Assessment Applications

- Prioritization
  - Screening, testing, and decision-making (e.g. HPV Challenge, EDC, CCL)
- Reporting Provisions
  - How genomics information triggers reporting requirements, right-to-know provisions (e.g. TSCA 8(e), FIFRA 6(a)(2))
- Risk Assessment Applications
  - Identify possible mode(s) of action
    - Hazard; Dose-response assessment; Interspecies, High to low dose extrapolations; Exposure assessment
  - Mixtures assessments
- http://www.epa.gov/osa/genomics.htm


U.S. Environmental Protection Agency

Potential Implications of Genomics for Regulatory and Risk Assessment Applications at EPA

Interim Guidance for Microarray-Based Assays

- **The Draft Interim Guidance document provides guidance to:**
  - **Regulated community and other interested parties regarding submitting microarray data to the Agency**
  - **EPA reviewers evaluating such information**

- **Interim Guidance is expected to be used by EPA program offices to determine the applicability of specific genomics information to the evaluation of chemical risks**

- **http://www.epa.gov/osa/spc/genomicsguidance.htm**

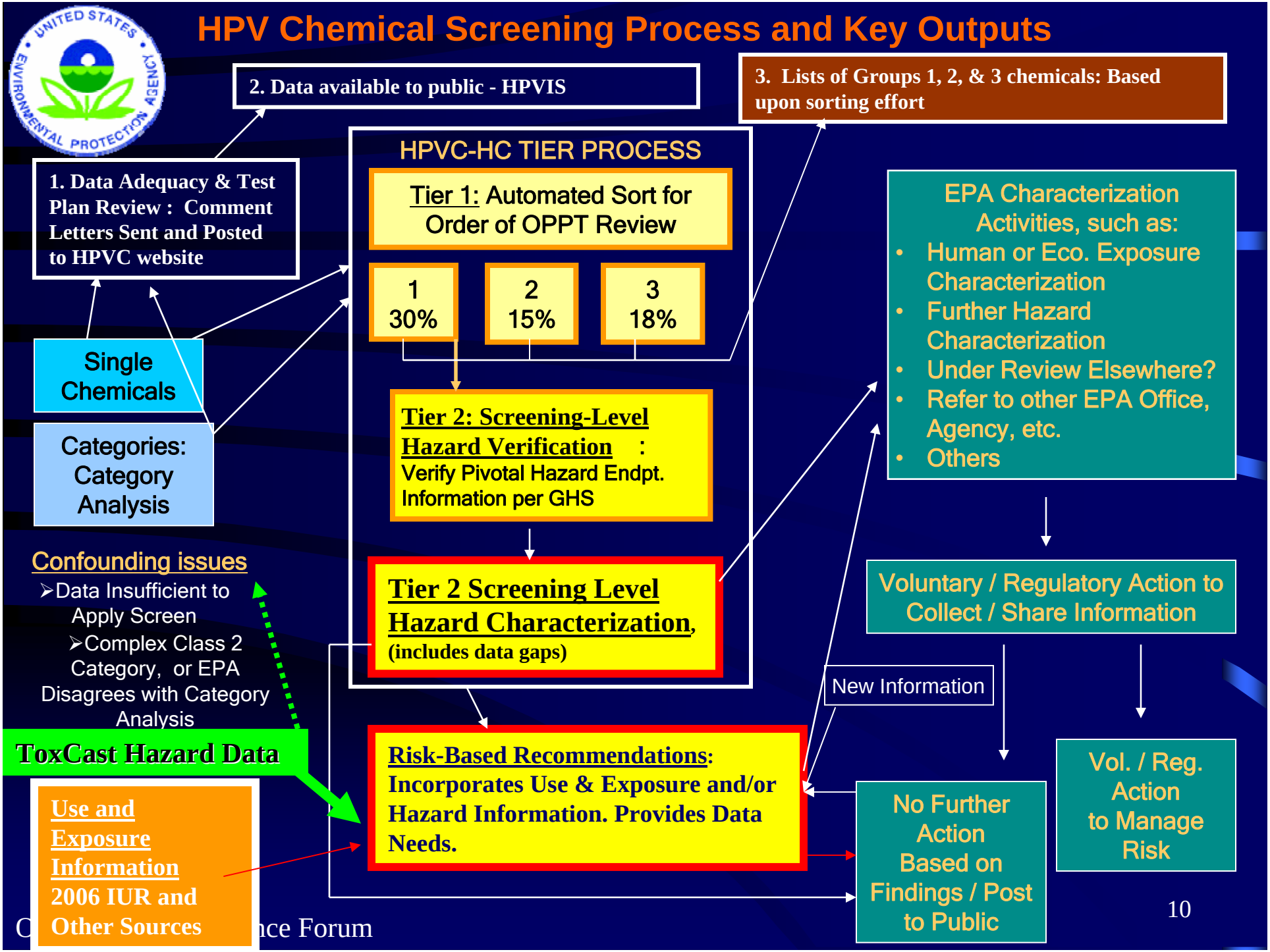
EPA HPV Challenge, and OECD SIDS

- Single chemical or Category
  - Chemical analog data often used to fill data gaps
  - Approximately 80% of HPV Challenge chemicals are in Categories
- OECD SIDS Endpoint data on approximately 800 HPV Challenge chemicals @ **http://www.epa.gov/hpvis/**
- Data on over 500 OECD SIDS HPVs is available **http://cs3-hq.oecd.org/scripts/hpv/**
- Test Plan & Analog Justifications (if applicable)
  - Category Guidance (http://www.oecd.org or http://www.epa.gov/chemrtk/)
- Endpoints of Interest
  - Physicochemical Properties, Environmental Fate, Ecological Effects
  - **Health Effects: acute and subchronic toxicity, genetic toxicity, reproductive and developmental toxicity**
- Robust Summaries of tests conducted and results

Hazard Characterization
Tier 1 Screening – Human Health

- **Endpoints:** Repeat Dose Toxicity (primary), Genetic toxicity (both gene mutation and chromosomal aberration), Reproductive toxicity, Developmental toxicity
- **Step 1** – Apply Globally Harmonized System (GHS) criteria to **Repeated Dose Toxicity LOAEL**
  - Review Group 1: GHS Category 1
  - Review Group 2: GHS Category 2
  - Review Group 3: Does not meet GHS Category 2 criteria
- **Step 2 – Modifying Factor 1: Reproductive and Developmental Toxicity**
  - Upgrade the chemical to next higher group if:
    - the reproductive or developmental effect is observed at much lower doses than the repeat dose effect for a chemical that is placed in review Group 3 in Step 1
    - either the reproductive or developmental LOAEL is within the range of the GHS Category 1 criteria, the chemical is upgraded into the review Group 1 for human health.
- **Step 3 – Modifying Factor 2:** For chemicals placed in Group 2 or Group 3 in Steps 1 and 2, if one or more endpoints among genetic, reproductive, or developmental toxicity are positive, the chemical is upgraded into the next highest review group.

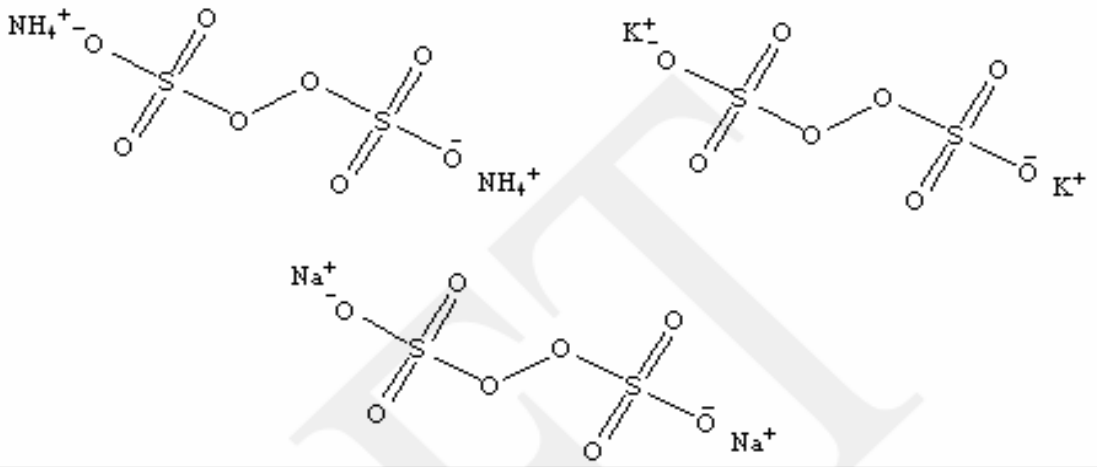
HPV Chemical Screening Process and Key Outputs



ToxCast and Industrial Chemicals

- **Insights into Mode of Action / Support for endpoint concerns for individual chemicals, or those in a category**
  - Reproductive Toxicity
  - Developmental Toxicity
  - Organ, and other, toxicities
- **Utility in assessing an HPV** chemical for Tiering in absence of full SIDS data set
- **Addressing other data gaps for HPVs**
  - Cancer
  - Neurotoxicity
  - Pulmonary Toxicity
  - Immunotoxicity
- **Expansion of existing chemical categories (HPV, MPV, others)**

ToxCast & Categories: Persulfates OECD HPV Category

Chemical Name	Ammonium persulfate, potassium persulfate, sodium persulfate
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Category Rationale

The persulfates category includes molecules with similar chemical structure and similar physical-chemical properties. The inorganic substances differ only by the cationic portion of the salt, which is not expected to influence the hazardous properties of the molecule. The anionic part is identical and, therefore, the three salts are expected to display the same environmental, ecotoxicological and toxicological behaviour based on the available data.

Read Across Conclusions for Several Health Endpoints Could be Supported by ToxCast Findings

	Ammonium persulfate CAS 7727-54-0		Potassium persulfate CAS 7727-21-1		Sodium persulfate CAS 7775-27-1	
	Value	Comment	Value	Comment	Value	Comment
Ames Test	negative	review article		Read across	negative	
UDS					negative	
Chrom. Aberration	negative			Read across		
Genetic toxicity in vivo						
Micronucleus				Read across	negative	
Unscheduled DNA					negative	
Subchronic/Reproduction						
28 day (NOAEL)	41.1 mg/kg bw/day		131.5 mg/kg bw/day		137.2 mg/kg bw/day	
90 day (NOAEL)	10.3 mg/m3	inhalation		Read across	LOAEL = 3000 ppm	= 200 mg /kg bw/day (M*); 250 mg /kg bw/day (F*)
Reproduction toxicity (NOAEL)	250 mg/kg bw/day			Read across		Read across
Developmental toxicity (NOAEL)	250 mg/kg bw/day			Read across		Read across

Based on Avail Info, no Evidence of Toxicity

ToxCast Data Use in Conjunction with the Analog Identification Methodology (AIM)

- The AIM database contains 31,031 potential analogs with publicly available toxicity data
- Enter chemical by CAS, SMILES, or Drawing structure
- Experimental data sources indexed
  - On-Line Databases
    - TSCATS, HSDB, IRIS, RTECS*
  - U.S. Government Documents
    - NTP, ATSDR, HPV Challenge Program
  - Other Sources
    - DSSTox, RTECS, IUCLID, AEGLS
- Uses a chemical fragment-based approach with 645 individual chemical fragments to identify potential analogs of organic compounds.

Generation of new/expanded chemical groupings with AIM

- **Over 1900 HPVs with discreet or representative structures**
- **Run individual chemicals against all others in database → new/expanded candidate clusters**
- **Do Clusters align with existing HPV categories?**
- **Do ToxCast data align with new/ expanded clusters for endpoints of concern?**
- **Selection of new ToxCast chemicals based on AIM identification of analogs with test data**

USEPA National Pesticide Program

- Programmatic challenges
  - **Over 5,000 regulatory decisions annually**
    - **actives, antimicrobials, inerts**
  - **Data availability/quality varies extensively**
  - **Science increasingly complex & changing**
    - **FQPA issues (mechanism of toxicity, susceptibility, cumulative effects)**

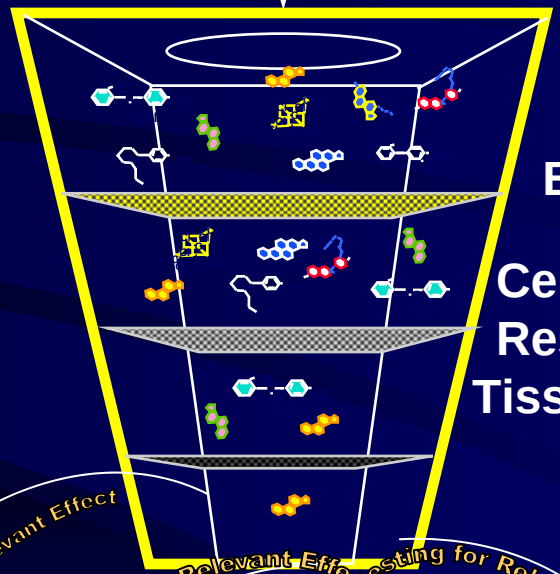
Current Toxicology Testing Paradigm

- Current paradigm generates lab animal data for all possible outcomes to determine which of all possible effects are relevant

The New Hypothesis-Driven Paradigm
Vision for a New Toxicology Testing & Assessment Paradigm

Existing data;
In silico and
In vitro
Prioritization;
Screening

Pesticide Inventories



Molecular Interactions

Biochemical Responses

Cellular Responses

Tissue/Organ Function

ToxCast

Virtual Liver

Efficient, Focused
In vivo Animal Testing

Testing for Relevant Effect

Testing for Relevant Effect

Testing for Relevant Effect

Adverse Outcomes

Mortality
- Systemic Toxicity
- Disease
- Cancer

Reproductive Fitness
- Viable Offspring
- Fertility

Developmental Impairment
- Terato
- Prenatal Deficits

Tiered Testing Paradigm – A more efficient approach to animal testing

2006 Agricultural Chemical Safety Assessment Proposal by ILSI/HESI

Existing Knowledge/Data
(e.g., ToxCast/ToxRef, Virtual Liver, discovery studies, other available data)

Base Set of Animal Tox Studies

Enhanced F1 lifestage study
Subchronic dog & rat studies
etc

triggers

Higher tiers of testing if need to further evaluate special toxicities or mechanisms

ToxRef/ToxCast: Benefit to the Risk Assessment Process

- **Incorporate Lessons Learned into development of a New Testing Paradigm for Regulatory Practice**
- **Build capacity (via QSAR, *in silico*, *in vitro* methods, "omics" technology, etc) to prioritize, screen & evaluate chemicals by enhancing the predictive understanding of toxicity pathways**
- **Assist in grouping common mechanism pesticides for cumulative risk assessments (as required by the 1996 Food Quality Protection Act)**
- **Assist interpretation of data**

Examples of EPA Assessments that have Evaluated Genomics Data

- Genomics data have been used to corroborate Modes of Action for critical effects:

  Dibutyl Phthalate, PFOA, Acetochlor

- Ongoing research at EPA's ORD is using 'omic technologies to identify key changes in toxicity pathways for Conazoles